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Review article

Emerging and re-emerging rickettsial infections

Patricia V. Adem

Department of Pathology, New York Medical College School of Medicine, 30 Sunshine Cottage Road, Basic Sciences Building, Room 413, Valhalla, NY 10595, United States

A B S T R A C T

Rickettsial organisms are a diverse group of obligate intracellular bacteria; all species known to cause human disease are dependent on an arthropod vector and many are considered zoonotic diseases. Typical vectors of rickettsia are fleas, ticks, mites or lice. Humans become infected either when bitten or upon contact of broken skin or mucous membranes by infected secretions from an arthropod vector. The emergence and re-emergence of rickettsial diseases is a serious public health concern in the United States and abroad. Herein, the clinical and pathologic features of rickettsial diseases are described in tandem with the current scientific underpinnings. The histopathology of emerging and re-emerging rickettsiosis with species-specific discussion relating to vector issues and control are explored. Concepts of endemicity are addressed in the context of climate change and its impact on vector and sylvatic reservoirs, underscoring the need for clinical vigilance and broad consideration for encounters with these potentially life threating human pathogens.

Rickettsia as endosymbionts

The reductive nature of arthropod genomes provides some evidence that the rickettsiae are true endosymbionts (or endobiont; any organism that lives within the body or cells of another organism in a mutualistic –formerly called symbiotic- relationship with the host body or cell, often but not always to mutual benefit), providing cellular machinery missing from their host's genome. The benefit to the arthropods is evident in studies whereby both arthropod fecundity and survival are improved.[1,2] Thus, Rickettsia are appropriately considered intracellular endosymbionts carried in a variety of arthropod tissues; those causing disease are transmitted to humans via bite or contact with secretions from an infected arthropod. It is impossible to consider rickettsiae without an appreciation of their arthropod vectors and applicable vertebrate reservoirs.

To better understand the impact of rickettsial pathogens in humans requires a broader awareness beyond that encompassing the known rickettsial organisms and their conventional vectors. The *Rickettsia* genus has two ancestral clades, one branch carried by arthropods and the second branch carried by other eukaryotes.[3] In fact, a large body of literature is emerging regarding the discovery of rickettsial carriage among arthropods that are not hematophagus ectoparasites. Up to 24% of terrestrial arthropods carry rickettsial endosymbionts.[4] For example, the parthenogenically-reproducing booklouse *Liposcelis bostrychophila* carries *Rickettsia felis*, a spotted-fever group rickettsia species typically seen in fleas.[5]

Arthropod acquisition of rickettsia occurs by two mechanisms. Arthropod carriage of rickettsia may be transstadial (when a pathogen remains with the vector from one life stage –"stadium"- to the next); that is, horizontally carried from one stage of the arthropod life cycle to the next commencing upon consumption of a blood meal from an infected host animal. Rickettsia may also be propagated in an arthropod species by way of transovarial transmission (or transovarian transmission occurs in certain arthropod vectors as they transmit disease-causing pathogens from parent arthropod to offspring arthropod) from infected arthropod mother to her progeny. Clearly, the carriage and propagation of rickettsia within arthropod hosts confers selective advantage and typically endosymbionts are either obligate or facultative.

The advent of newer genome sequence technology enables survey of many new potential hosts and the identification of new, yet uncultured, rickettsia, thus we have seen an expansion of the rickettsia genus as a result.[6] In a similar vein, the complex relationships between the rickettsias and their respective arthropod and mammalian hosts' microbiomes are just beginning to unfold. A recent review explored the tick gut microbiome modulation of pathogen carriage which is dynamic and changes in response to a number of factors including life cycle stage, feeding status, and host animal interactions among others.[7]

While the *Rickettsia* genus is adapted to a wide variety of host organisms, the respective species may be broad or restrictive in their vector host or hosts as applicable.[8] The Anthropocene era has impacted the prevalence and distribution of rickettsial diseases by a number of mechanisms that ultimately create greater opportunity for human-vector interactions.[9] Warmer wetter climates have led to the expansion of tick habitat range and distribution well documented for *Dermacentor reticulatus*.[10] This has led to the concomitant emergence *Rickettsia roultii*, one of the agents of scalp eschar and neck lymphadenopathy (SENLAT) also known as *Dermacentor* borne necrosis erythema lymphadenopathy/ tick-borne lymphadenopathy (DEBONEL/TIBOLA) in the same geographic area.[11] The expansion of the lone star tick geographic range was recently correlated with an increased incidence of

E-mail address: patricia.adem@wmchealth.org.

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spotted fever group rickettsiosis in the United States.[12] Migratory birds carry both ticks and arthropod-borne diseases and may introduce them both to entirely new habitats and hosts.[13–15]

Rickettsial pathogenesis in humans

Rickettsia are small gram-negative bacteria with an obligate intracellular lifestyle attributed to the loss of metabolic and synthetic pathways necessary for extracellular life. Rickettsias, due to their extreme virulence in humans, require biosafety level −3 (BSL-3) for proper containment. Mainly as a result of the fastidious nature of the organism, culture of rickettsias is difficult and requires highly specialized laboratory settings proficient with cell line culture methods necessary to propagate them in vitro.[16] The exact mechanisms by which rickettsia exert their pathogenic effects on humans remain elusive, at least in part due to the fact that laboratory animals demonstrate little to no pathology when infected with rickettsias and limited data can be gleaned via in-vitro tools. Genomic assessment of human rickettsial pathogens, in contrast with closely related non-pathogens, shows no evidence of acquisition of novel virulence genes. Defective or deficient replication systems may be underpinning virulence.[17] There is a paradoxical inverse relationship between rickettsial genome size and human pathogenicity, as smaller rickettsial genomes are found in more pathogenic rickettsia.[18]

During human infections rickettsia disseminate to endothelial cells which become dysfunctional and lose integrity. Both microvascular thrombosis and increased permeability of microvasculature with resultant edema are common histologic findings in rickettsial infection. The mechanisms of rickettsia-mediated human pathophysiology have been studied in-vitro utilizing human umbilical vein endothelial cells (HUVECs) and human dermal microvascular cells. Internalization of spotted fever group rickettsia into the endothelium was recently demonstrated to be mediated by fibroblast growth factor receptor-1.[19] HUVECs undergo a number of pathogenic changes when infected with rickettsia, expressing tissue factor[20] and releasing the high molecular weight multimers of von Willebrand factor[21] both of which are essential components of normal coagulation and may account in no small part for thrombosis in the microvasculature associated with spotted fever group rickettsial infections. Furthermore, rickettsia infected HUVECs demonstrated E-selectin dependent neutrophil adhesion[22] which could result in tissue infiltration and inflammation seen with human rickettsial infections. Recent proteomics studies with *Rickettsia conorii* infected HUVECs revealed activation of the JAK-STAT and interferon pathways in contrast to HUVECs incubated with LPS; rickettsia-infected HUVECs display a pattern of cellular derangements which would impair both endothelial cellular adhesion and antigen presentation functions in vivo.[23]

Human rickettsial disease: serologic and genomic categorical consideration of spotted fever group rickettsias, transitional group rickettsias, and typhus group rickettsias

Adapting to a wide variety of hosts ranging from vertebrates, invertebrates, and others, is a hallmark of *Rickettsia* over the course of their evolution.[3,17] Human rickettsial pathogens vectored by hematophagus arthropods presented more recently in the phylogentic history of the genus.[3,17] Prior to the advent of molecular techniques, rickettsial pathogens were separated into three families on the basis of human serological responses: spotted fever group, typhus group, and scrub typhus group. Scrub typhus was previously considered part of the *Rickettsia* genus; sequencing data has enabled the identification of *Orentia* as a separate genus.[24] Thus, *Orentia* merits only this brief mention due to the historical inclusion in the *Rickettsia* genus, otherwise *Orentia* is outside of the scope of this manuscript. Table 1, adapted from a recent review highlights confirmed vector-borne rickettsiosis in North America.[25] Transitional group rickettsias are those belonging to the

Table 1
Confirmed vector-borne human rickettsiosis in North America.

Rickettsial organism	Disease	Confirmed Primary Arthropod Vector(s)	Vector Host(s)	Distribution	Primary Clinical Manifestations
R. rickettsii	Rocky Mountain spotted fever	Ticks: *Amblyomma* species, *Dermacentor* species, *Rhipicephalus sanguineus*	Small mammals, companion animals	Southeastern and southwestern U.S.; Mexico	Fever, headache, myalgia, malaise, rash (typically not on palms and soles)
R. parkeri	Maculatum infection, Tidewater spotted fever, American boutonneuse fever	Tick: *Amblyomma maculatum*	Mammals, birds	Southern portions of U.S.	Fever, headache, malaise, myalgia/arthralgia, eschar, maculopapular rash
364D	Pacific Coast tick fever	Tick: *Dermacentor occidentalis*	Rodents, companion animals, urban wildlife	California U.S.	Fever, headache, eschar, maculopapular rash including palms and soles
R. felis	Flea-borne spotted fever	Flea: *Ctenocephalides felis*	Companion animals, urban wildlife	Southern California, Texas, Hawaii U.S.; Mexico	Fever, headache, rash
R. akari	Rickettsialpox	Mite: *Liponyssoides sanguineus*	House mouse, other rodents	Major urban centers	Fever, eschar, papulovesicular rash
R. typhi	Murine typhus, endemic typhus	Fleas: *Xenopsylla cheopis, Ctenocephalides felis*	Rodents, companion animals, urban wildlife	Southern California, Texas, and Hawaii U.S.; Mexico	Fever, headache, maculopapular rash including palms and soles
R. prowazekii	Epidemic typhus, sylvatic typhus	Lice: *Pediculus humanus corporis*, Southern flying squirrel ectoparasites	Humans, flying squirrels	Eastern half of U.S.	Fever, eschar, headache, lymphadenopathy

Adapted from Hardstone Yoshimizu M, Billeter SA. Suspected and Confirmed Vector-Borne Rickettsioses of North America Associated with Human Diseases. Tropical Medicine and Infectious Disease. 2018; 3(1):2.doi:10.3390/tropicalmed3010002.

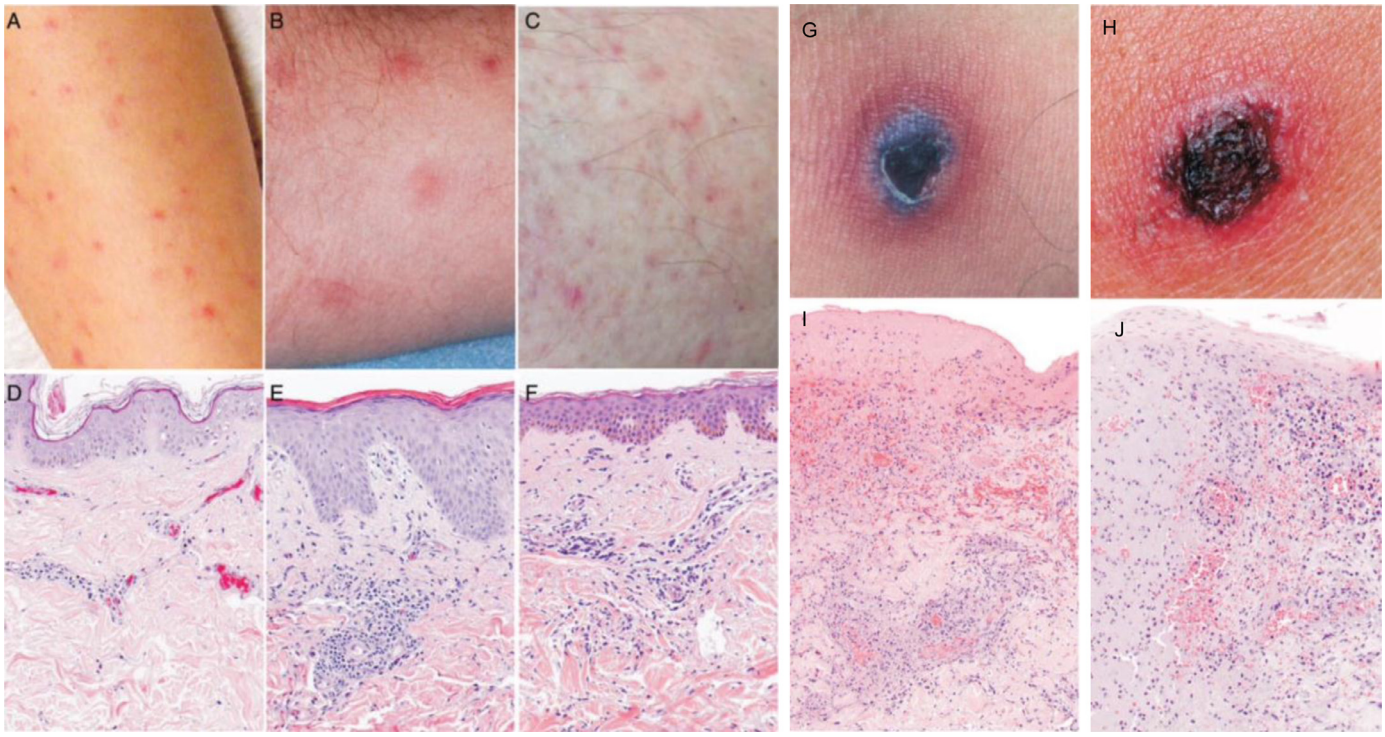

Fig. 1. Maculopapular rashes caused by spotted fever group rickettsias (SFGR) are clinically and histologically identical with prominent perivascular mononuclear infiltrates. Likewise, the eschars produced by SFGR are both clinically and histologically identical, featuring mononuclear perivascular infiltrates in the deep dermis, and microvascular fibrin thrombi induced ischemic necrosis of superficial dermis with loss of epidermis.
R. rickettsii (A and D), R. parkeri (B and E, H and J), R. akari (C and F, G and I), Photomicrographs: hematoxylin and eosin, 25x
Image adapted from Denison AM, Amin BD, Nicholson WL, Paddock CD. Detection of Rickettsia rickettsii, Rickettsia parkeri, and Rickettsia akari in skin biopsy specimens using a multiplex real-time polymerase chain reaction assay. Clin Infect Dis. 2014;59:635–42.

spotted fever group serologically but possessing genomic overlap with typhus group rickettsias; these are spotted fever rickettsias with much milder clinical courses and no reported fatalities, such as *R. felis* and *Rickettsia. akari*.[26]

Clinical presentation and diagnosis

Clinically significant rickettsial infections generally present with fever, headache, and rash (Fig. 1A–C) with or without eschar (Fig. 1G and H); additional symptoms of myalgia and arthralgia may also be seen. When confronted with such symptoms clinicians must have a high index of suspicion for rickettsiosis and prompt empiric treatment with tetracycline antibiotic preferably doxycycline is indicated, given that the clinical course of rickettsial infections is highly variable ranging from self-limited in the case of *R. akari* to fulminant organ failure and death due to *Rickettsia rickettsii*. Definitive diagnosis of rickettsial diseases is most often achieved in retrospect via serologic methods when a four-fold increase in titer is observed. Biopsy of rash or affected tissues reveals mononuclear perivascular inflammation with or without microthrombi (Fig. 1D–F). When an eschar is present clinically, the tissues show necrosis of the epidermis and superficial dermis (Fig. 1I and J). Relatively rapid diagnosis of rickettsial pathogens is possible via direct detection of rickettsial antigens in lesional tissues, alternately a diagnosis may be achieved via real-time or conventional PCR testing of lesional tissues.[27] However, all tissue-based methods inevitably suffer from poor sensitivity, where sampling errors may miss lesions or patients without cutaneous findings could be neglected entirely.

The availability of rapid and reliable molecular blood tests for spotted fever group and typhus group rickettsial diseases remains elusive due to the limited bacteremic phase inherent to the rickettsial organisms.[28] Indirect immunofluorescence assay has been considered the gold standard, however this method has limited utility in species determination within a serogroup due to extensive cross reactivity. As of 2004, qualitative ELISA based methods enabled the identification of probable cases,[29] again, serogroup cross-reactivity remains a problem. Practically speaking, rickettsial testing outside of tissue based methods for clinical diagnosis is generally limited to reference laboratories or large academic settings due to the relatively high costs, regulatory burdens, and lack of standardization associated with laboratory developed tests.

Spotted fever group rickettsias (SFGR)

R. rickettsii

Rocky Mountain spotted fever (RMSF), whose causative agent it *R.rickettsii*, is the prototype of the spotted fever group rickettsias (SFGR) with widespread geographic distribution. In the U.S., many species of SFGR have been associated with variably severe human disease including: *R.rickettsii, Rickettsia montanensis, Rickettsia amlyommii,* and *Rickettsia parkeri*. The SFGR may have variable and overlapping clinical and pathological presentations (Fig. 1A–J), which when taken in conjunction with known serological cross-reactivity can confound their diagnosis and any subsequent categorical assessments of pathogen prevalence.

Clinical features of RMSF are fever, headache, myalgia, and rash. When patients present, they require prompt initiation of treatment with tetracycline or doxycycline to avoid organ failure and a rapidly fatal outcome. Most deaths occur before day 9.[30] Monitoring and reporting of RMSF cases provides important information to aid clinicians and epidemiologists, yet there are limitations to consider when looking at the passive surveillance data. Due to the limitations of rickettsial testing described previously, currently most reported cases are considered probable on the basis of clinical criteria and a single serologic result.[31]

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Criteria for confirmation of cases includes: paired serology, culture, PCR, and immunohistochemistry of tissue.

Between 2000 and 2007 probable RMSF cases and incidence per million increased while both the case fatality rate and percent confirmed cases declined.[32] This pattern was identical through 2012.[33] The RMSF surveillance category was renamed Spotted Fever Rickettsiosis in 2010 due to the emergence, clinical identification, and inclusion of SFGR other than RMSF in the surveillance data for probable RMSF cases.[31,34,35]

Despite reassuring epidemiological data from the U.S., fatal RMSF cases remain a problem particularly in the southwest and at the U.S.-Mexico border. In the southwest, populations of RMSF tick vectors such as *Rhipicephalus sanguineus* sensu lato are responsible for RMSF transmission[36–38] and for fatal cases.[39] Also noteworthy is that children continue to have a higher case-fatality rate compared to adults,[33] possibly due to physicians' reluctance to prescribe tetracycline antibiotics to children due to concerns for dental enamel staining.

R. parkeri

In 2004, the first definitive case of spotted fever rickettsiosis due to *R. parkeri* was published.[40] *R. parkeri* infections are typically vectored by the Gulf Coast tick, *Amblyomma maculatum* in the U.S. The clinical symptoms of *R. parkeri* rickettsiosis include multiple eschars, maculopapular rash, fever, headache, myalgias, arthralgias, and malaise with subsequent development of maculopapular rash. The clinical features of this emerging rickettsiosis were further elucidated in a subsequent case series of twelve patients[41] which supported the initial report. Symptoms of *R. parkeri* resemble RMSF but without significant morbidity and zero mortality. As the vector habitat range expands in the U.S.,[42] the identification of illness due to *R. parkeri* will inevitably expand as well.[43]

Rickettsia 364D

In 2010 the first report of human disease associated with *Rickettsia* 364D was published, where patients had developed eschar without additional symptoms.[44] Subsequently, Pacific Coast tick fever has been proposed as the name for 364D-associated rickettsiosis.[45] Pacific Coast tick fever has a milder clinical course than RMSF, limited cutaneous findings typically one or more eschars with relatively rare generalized rash. *Rickettsia* 364D has been published in the literature, inappropriately under the name *Rickettisa philipii,* outside of the accepted standards.[6] 364D has not as of yet met species requirements[46] and it remains to be determined if *Rickettsia* 364D represents a unique species.

R. felis

R. felis is also known as flea-borne spotted fever, first identified as a human pathogen in 1991.[47] This organism has been identified in cat fleas (*Ctenocephalides felis*) as well as many non-hematophagus arthropods.[4,5] Clinical infection with *R. felis* is thought to be acquired via bites. Typical presentations include fever, headache, and myalgia, with or without rash. PCR has demonstrated little efficacy in detecting rickettsias in the blood, the sole exception to this rule is *R. felis.* Detection of *R. felis* by PCR in patients with and without fever[48,49] raises the possibility that humans may be a natural reservoir for *R. felis,* with speculation that patients may recrudesce intermittently in a manner similar to Brill-Zinsser disease.[50] In contrast some authors conclude that there is insufficient evidence that *R. felis* is the causative agent of fever in Africa.[51] Additional studies will be necessary to elucidate the pathogenicity of *R. felis.*

R. akari

R. akari is the etiologic agent of rickettsialpox, an illness first recognized by Huebner in 1946.[52] This rickettsiosis has been identified in many urban settings in the U.S. and abroad, and remains a notifiable condition in New York City.[53] The clinical presentation is an eschar at the site of inoculation via bite from the house mouse *Mus musculus* mite *Liponyssoides sanguineus.* About a week subsequent to the bite an eschar forms, then patients develop febrile illness with headache and papulovesicular rash. Rickettsialpox is typically a self-limited infection resolving after one to three weeks. Administration of tetracycline or doxycycline is recommended to ensure prompt resolution of symptoms.

Typhus group rickettsias

Rickettsia tyhpi

R. typhi is the rickettsial pathogen causing murine typhus also known as endemic typhus or flea-borne typhus. The majority of the murine typhus diagnosis in the U.S. is identified in Texas, California, and Hawaii. Increased number of cases reaching outbreak proportions have been identified in California from the end of 2018 to 2019.[54,55] *R. typhi* is maintained in urban and suburban sylvatic animal reservoirs in the warm coastal regions of the U.S., where urban reservoirs are rats, and suburban reservoirs are opossums, cats, and other small mammals. Murine typhus is not a nationally notifiable condition. National prevalence data and unified diagnostic criteria do not exist. At present murine typhus remains reportable in 14 states, and each state defines their own diagnostic criteria for probable and confirmed cases. Upon being bitten by an infected rat flea (*Xenopsylla cheopsis*) or cat flea (*Ctenocephalides felis*) flea feces may be rubbed into a wound, introducing *R. typhi* to the bloodstream. Once in the bloodstream *R. typhi* disseminates to endothelium to cause what is typically a self-limited illness, but which may be fatal in up to 4% of untreated cases.[56] Typical symptoms present within 6–14 days of exposure. The clinical and epidemiological features of eleven cases of fatal murine typhus reported from Texas between 1985 and 2015 were recently reviewed.[57] Clinically most of these patients present with fever, headache, myalgia, rash, anorexia, nausea, vomiting, and respiratory complaints including cough, pneumonia, pulmonary edema, and acute respiratory distress syndrome. Laboratory features include thrombocytopenia and elevated transaminases. These symptoms may be clinically indistinguishable from those associated with epidemic typhus caused by *Rickettsia prowazekii. R. typhi* was recently identified via PCR as the causative agent of encephalitic typhus in archival formalin fixed paraffin embedded brain tissue from autopsies performed in Hamburg Germany between 1940 - 1944, indicating that at least some wartime deaths historically ascribed to *R. prowazekii* may in fact reflect disease due to *R. typhi.*[58]

R. prowazekii

Diseases caused by *R. prowazekii* include epidemic typhus and the typically milder Brill-Zinsser disease. In epidemic typhus, the vector for *R. prowazekii* is the human body louse *Pediculus humanus corporis*, which thrives during war times and whenever hygiene is compromised. Infection is achieved by rubbing louse feces or louse parts into wounds or mucous membranes, and may also be acquired by inhalation. In contrast to arthropod symbiont rickettsias which may confer survival benefit, there is no maintenance of *R. prowazekii* infection in lice. Upon ingestion of *R. prowazekii* the lice gut epithelium becomes infected and loses integrity. As the blood meal leaks out the lice turn red, and within one week they are dead. It is vertebrate hosts that maintain *R. prowazekii*[59]

Sylvatic epidemic typhus cases are vectored by the hematophagus ectoparasites of the flying squirrel *Glaucomys volans*, which is found in the eastern half of the U.S. Although typically associated with the southeastern states two cases associated with flying squirrels were identified in New York State.[60] Disease caused by *R. prowazekii* is nationally notifiable due to its potential utility as a bioterror weapon. The high case fatality index if untreated, lengthy stability of the pathogen in

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dried louse feces, and the potential for inhalation of infected louse fecal dust as a portal of entry make this pathogen particularly dangerous. Subclinically infected patients are at risk of recrudescence during times of stress and may develop Brill-Zinsser disease, which may present in patients without recollection of primary infection.[61] A case report raises the intriguing possibility that a lag in the administration of appropriate tetracycline antibiotics relative to acute symptoms may render patients susceptible to Brill-Zinsser disease.[62]

Conclusion

Rickettsial organisms are a diverse group of bacteria with variable pathogenicity. Their pathogenesis and the relationships with their arthropod hosts is not entirely known. All clinically significant rickettsiosis disseminate to infect endothelium where they adversely impact vessel integrity and promote microvascular thrombosis. Substantial problems exist in the determination of the extent of disease attributable to respective rickettsial species, due to both clinical and serological overlap among the species, difficulties in culture, and the limited utility of molecular techniques on whole blood. Patients presenting with fever, headache, rash with or without eschar(s), and myalgias must raise a high index of suspicion for rickettsiosis, requiring tetracycline-class antibiotic therapy to avoid potentially fatal consequences. Rickettsial disease may come to the attention of the pathologist subsequent to biopsy of a rash lesion, where the histologic features, clinical history, and travel history may help inform the diagnosis.

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